

## GENERAL NOTES

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### A "MICROBIAL COST" OF BUTTERFLY-ANT MUTUALISMS (LYCAENIDAE)

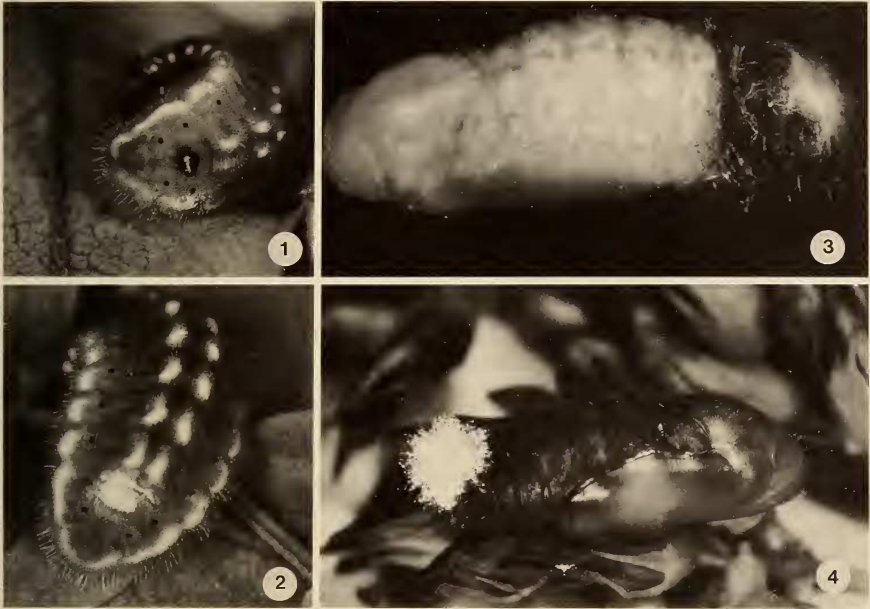
**Additional key words:** *Polyommatus*, Formicidae, myrmecophily, dorsal nectary organ, fungal infection.

The larvae of a large number of species in the Lycaenidae and Riodinidae associate with ants (Fiedler 1991). These associations, termed myrmecophily, are mediated by secretions of specialized epidermal glands, so-called myrmecophilous organs (Cottrell 1984). Vibratory communication between caterpillars and ants also may be involved (DeVries 1991). In certain cases, such as the well known Palearctic genus *Maculinea* van Eecke, larvae enter the host ants' nest at some stage of their development, where they prey on ant brood or solicit trophallactic feeding from their hosts (Thomas et al. 1989). Such ant-butterfly interactions bear traits of parasitism. In contrast, the majority of Lycaenidae associate with ants outside ant nests on their hostplants (Fiedler 1991). These caterpillars attract ants with the help of nutritive secretions from two types of glands. The dorsal nectary organ (DNO), located mediodorsally on the seventh abdominal segment, secretes droplets of a clear liquid containing carbohydrates and/or amino acids (Maschwitz et al. 1975, Pierce 1983, 1989). In addition, numerous minute hair-derived glands called pore cupola organs (PCOs) deliver secretions attractive to ants, probably amino acids in certain cases (Pierce 1983). Taken together, these secretions may constitute a substantial food resource for ants (Pierce et al. 1987, Fiedler & Maschwitz 1988). The ants, in turn, may protect the caterpillars against natural enemies (Pierce & Eastal 1986, Pierce et al. 1987). Hence, butterfly-ant interactions of this latter type are mutualistic trophobioses (but see Peterson 1993).

Mutualistic interactions between different species generally are accompanied by trade-offs between costs and benefits for both partners. The delicate and complex balance between costs and benefits is decisive for the ecological outcome and evolutionary stability of any such interspecific association. In lycaenid butterfly larvae, the production of energy-rich secretions may pose a severe developmental cost (Pierce et al. 1987, Baylis & Pierce 1992; but see Fiedler & Hölldobler 1992, Wagner 1993, Fiedler & Saam 1994). Furthermore, parasitoids may be attracted by the ant-guard (Nash 1989) or by the DNO secretions (Schurian et al. 1993). We here report on another type of cost associated with myrmecophily—fungal infections that invade the larvae via the DNO.

In summer 1992, we reared larvae and pupae of 3 myrmecophilous European lycaenid species in association with ants to investigate possible developmental costs of myrmecophily (Fiedler & Saam 1994). All larvae were reared from eggs laid by field-caught females. Caterpillars were kept individually in closed translucent plastic vials (125 ml) from the third instar onwards. The vials were lined with moist filter paper and were placed in an environmental chamber (25°C, L:D cycle 16:8 h). Food in excess (freshly cut plant material from a natural habitat) was provided daily, larval faeces were removed daily, and each caterpillar was transferred into a clean plastic vial with new filter paper every day. Hence, although the rearing conditions may have been more humid than in a natural environment, care was taken to reduce the risk of larval infections (see Schurian 1989, Fiedler & Saam 1994, for further details on the rearing method).

Most of the caterpillars were reared in association with worker ants taken from laboratory colonies of 3 different ant species (see below). One or more forager ants (depending on the treatment, see Fiedler & Saam 1994) were carefully placed in the rearing vial with a brush. The ants rapidly associated with the larvae and tended them constantly. The ants were moved daily into the fresh clean vials together with "their" caterpillar



FIGS. 1-4. Infected larvae and pupae. 1, Fourth (= final) instar caterpillar of *Polyommatus (Lysandra) bellargus*, with fungal infection at the DNO. White sporangia start to protrude from the glandular opening; 2, Advanced stage of fungal disease in *P. bellargus*. The whitish fungus covers an increasing portion of the dorsum of A7; 3, Freshly molted pupa of *P. (Lysandra) coridon*. The cast cuticle of the fourth instar larva is colonized by a fungus in the region of the former DNO. The pupa survived after removal of the cast skin (courtesy M. Treidtel); 4, Pupa of *Polyommatus icarus* with well developed fungus sporangia at the location of the larval DNO.

and remained with this caterpillar until pupation. In a few cases when ants died prior to pupation, these were replaced by nestmates to ensure a constant tending level.

Fungal infections starting at the DNO occurred in two facultatively myrmecophilous species: *Polyommatus icarus* (Rottemburg) reared on *Medicago sativa* L. (Fabaceae) inflorescences, and *P. (Lysandra) bellargus* (Rottemburg) reared on *Coronilla varia* L. (Fabaceae) leaves. The first external sign of infection was a dark discoloring at the DNO. One or two days later, the first whitish fruit bodies of the mold became visible (Fig. 1), and in the following 2-3 days the fungus extended widely around the DNO (Fig. 2). In total, three of 105 individuals of *P. bellargus* and 6 of 133 *P. icarus* caterpillars died from such infections in the final larval instar. In one *P. icarus* individual, the lethal fungus developed in the freshly molted pupa exactly at the location of the larval DNO (Fig. 4).

*Polyommatus icarus* and *P. bellargus* generally were quite susceptible to larval infections, which accounted for a mortality of 29 individuals (27.6%) in *P. bellargus* and 26 individuals (19.5%) in *P. icarus*, respectively ( $\text{Chi}^2$  1 df = 2.16,  $p = 0.14$ ). In a third, facultatively myrmecophilous species, *Plebeius (Aricia) agestis* (Denis & Schiffermüller) reared on *Geranium molle* L. (Geraniaceae) foliage, fungal infections at the DNO have not been observed, and the overall susceptibility of larvae to infectious diseases was significantly lower (3 out of 118 caterpillars, 2.5%, under identical rearing conditions;  $\text{Chi}^2$  1 df = 22.98;  $p < 0.0001$  for comparison *agestis* versus *bellargus/icarus*).

Surprisingly, all 9 caterpillars that died from a DNO-based fungal infection during summer 1992 had been kept in continuous association with ants throughout the third and fourth instar. The 3 caterpillars of *P. bellargus* had been associated with 5 *Lasius flavus* (Fabricius) workers, with 2 *Lasius flavus*, or with 2 *Lasius niger* (Linnaeus) ants, respectively. Out of the 6 *P. icarus* caterpillars, one had been kept with 5 *L. flavus* ants, one with 2 *L. flavus*, and two each with either 2 *L. niger* ants or 1 *Myrmica rubra* (Linnaeus) worker. Therefore, DNO-based fungal infections could develop irrespective of the species or number of tending ants. However, due to the small absolute number of such infections, their apparent restriction to ant-tended caterpillars was not significant statistically (Fisher's exact test,  $p > 0.32$  for individual *Polyommatus* species,  $p = 0.14$  for both species combined).

In 1991, a few cases of DNO-based fungal infections were observed in another European species, *Polyommatus (Lysandra) coridon* (Poda). As in the aforementioned lycaenids, *P. coridon* larvae are facultative myrmecophiles. In this case, however, the experimental caterpillars were collected in the field as third and fourth instars after hibernation, and then were kept without ants in the laboratory (rearing conditions as above). Infections developed in the fourth instar and usually killed the caterpillars. In one exceptional case, the infection broke out in the prepupal phase of the fourth instar (Fig. 3). This caterpillar successfully molted, and after removal of the fungus-covered exuvia, the pupa survived and eventually produced a healthy butterfly.

In his revision of the *Lysandra* group of the genus *Polyommatus*, Schurian (1989: 24) speculated that ant attendance could decrease the caterpillars' risk of suffering from bacterial or fungal infections, since certain secretions of ants (in particular from the metapleural gland) are well known for their antimicrobial properties (e.g., Hölldobler & Wilson 1990). We found no support for such a "hygienic effect" of ants on caterpillars. In the 1992 experiments, overall mortality rates due to larval infections were not different between ant-tended larvae (all ant treatments combined) and non-tended controls ( $\chi^2$  1 df  $< 0.11$ ,  $p > 0.74$  for *P. bellargus* and *P. icarus*).

In the case of DNO-based infections, remnants of the energy-rich secretions may have contaminated the cuticle and setae around the DNO, thereby facilitating the establishment of pathogenic fungi. Alternatively, the fungi could have germinated inside the glandular reservoirs of the DNO. In any case, the presence of a DNO with its opening into the larval body offers an additional opportunity for pathogenic fungi to attack lycaenid caterpillars. Although we have no data on the incidence of such infections under natural conditions, our observations indicate that this is another potential cost factor associated with myrmecophily, which previously has received little attention.

In the extensive literature on myrmecophilous Lycaenidae, there are scattered reports that the larvae of certain myrmecophilous lycaenids may suffer from infections if deprived of their host ants in captivity. However, most of these records lack quantitative data or illustrations, and all refer to obligatory myrmecophiles that produce large amounts of secretions and totally depend on their host ants for survival. Reported examples include 4 species in the predominantly African tribe Aphnaeini, namely *Aphnaeus hutchinsoni* Trimen, *Spindasis natalensis* (Westwood), *Chloroselas pseudozeritis* (Trimen), *Poecilmitis lycegenes* (Trimen), (Jackson 1937, Clark & Dickson 1971), as well as *Hypolycaena erylus* (Godart) and *Arhopala pseudocentaurus* (Doubleday) (Jacobson 1912, Norman 1949). Our findings in 3 European species of the genus *Polyommatus* show that infections at the myrmecophilous organs also occur in facultative myrmecophiles, and even if tending ants are present. Such larvae produce much smaller amounts of DNO secretions, less than 15  $\mu$ l per lifetime in the case of *P. icarus* (Burghardt & Fiedler unpublished data); they associate unspecifically with a variety of ant taxa; and they do not depend on the presence of ants for survival. Facultative myrmecophiles account for the species majority of the family Lycaenidae (Fiedler 1991). Clearly, the selective importance of infectious diseases in lycaenid caterpillars and possible trade-offs with myrmecophily are a rewarding area for further studies in the laboratory as well as in the field.

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LATENT POLYPHENISM AND DIRECT DEVELOPMENT IN  
*PIERIS VIRGINIENSIS* (PIERIDAE)

**Additional key words:** diapause, hostplant, Brassicaceae.

In many pierids, seasonal polyphenism in wing pattern is coupled with diapause (Oliver 1970, Shapiro 1976), which is facultatively inhibited by rearing larvae under long-day photoperiods and warm temperatures (*P. rapae* L., Barker et al. 1963; *P. napi* L., Lees and Archer 1981; *P. napi oleracea* Harris, Oliver 1970). Populations of *Pieris napi* may contain individuals with varying response to natural photoperiod (Shapiro 1976), as well as individuals whose diapause lasts for several years (Shapiro 1979). Unexpected environmental stress such as unseasonably cold weather can induce more individuals to enter carryover diapause, ensuring the population's survival during extreme but short-lived environmental changes (Shapiro 1979). Yet another pierid diapause pattern is one in which all members of the population diapause and the insect is univoltine.

*Pieris virginienensis* Edwards is a monophagous species that uses an ephemeral, vernal hostplant, toothwort, *Cardamine diphylla* (Michx.) A. Wood (= *Dentaria diphylla* Michx.). Matching the restricted growing season of its host, *P. virginienensis* is a univoltine species that enters diapause when reared at summer photoperiods that inhibit diapause in *P. napi oleracea* (Shapiro 1971). Facultative diapause has been observed in several *P. virginienensis* populations, however. Forbes (1960) refers to a rare second brood of *P. virginienensis* occurring in Massachusetts and West Virginia, but does not give specific localities. Bowden (1971) reared 3 non-diapausing offspring from a stock of *P. virginienensis* sent to him by S. A. Hessel presumably from Washington (Litchfield Co.), Connecticut.

Shapiro (1971) obtained direct-developing *P. virginienensis* by subjecting the larvae to continuous light at 25°C. While the normal adult phenotype of *P. virginienensis* resembles a smokey version of the heavily veined vernal form of *P. n. oleracea*, Shapiro found that non-diapausing *P. virginienensis* adults were indistinguishable from the summer form of *P. n. oleracea*, a phenotypic similarity also noted by Forbes (1960). Shapiro concluded that the mechanism for phenotypic polyphenism was still intact but latent in *P. virginienensis*. We report here on a population of *P. virginienensis* producing direct-developing individuals whose offspring we have crossed in the laboratory.

*Pieris virginienensis* occurs together with *P. n. oleracea* in beech-maple-hemlock woods near Lee, Massachusetts (Berkshire Co.). Large stands of toothwort are present in early spring and are followed by garlic mustard, *Alliaria petiolata* (Bieb.) Cavara & Grande (= *A. officinalis* Andr.), as the season progresses. By mid-July the toothwort population has senesced; only garlic mustard remains in abundance along with small stands of watercress, *Rorippa nasturtium-aquaticum* (L.) Hayek (= *Nasturtium officinale* R. Br.), and cuckoo-flower, *Cardamine pratensis* L. Together these crucifers could support a sizable summer brood of either species. Courant et al. (1994) present evidence of incipient host range expansion of *P. n. oleracea* to garlic mustard, but there is no evidence that *P. virginienensis* is able to exploit garlic mustard successfully (Bowden 1971, our unpublished data) although they oviposit on it in the field (Roger W. Pease, Jr. personal communication, Courant et al. 1994, Porter 1994).

On 7 May 1993 we collected from this site seven female *P. virginienensis* which laid